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Serial reversal learning in grey squirrels: learning efficiency as a function of learning and change of tactics.

ABSTRACT

Learning allows individuals to adapt their behaviours flexibly to changing environment. When the same change recurs repeatedly, acquiring relevant tactics may increase learning efficiency under recurring change. We examined this relationship, along with the effects of proactive interference and other interference information, in a serial spatial reversal task with five grey squirrels (*Sciurus carolinensis*). Squirrels completed an acquisition and 11 reversal phases with a poke box in which two out of four possible reward locations were baited diagonally in a square array. In this situation, an efficient tactic is to locate the diagonally related locations consecutively (integrative search tactic) instead of searching rewards in a clockwise or anti-clockwise direction (sequential search tactic). All the squirrels formed a learning set acquiring successive reversals in fewer trials. Although four individuals gradually employed more integrative tactics in locating the rewards both within and between phases, sequential tactics were used in the first trial of each phase. This suggests the integrative tactic did not depend on an association between the rewarded locations but was learned as a spatial pattern and/or by use of extra-apparatus cues to locate individual reward. Generalized Estimating Equation (GEE) models showed that learning efficiency increased with experience and tactic change. Although tactic change partially mediated the effect of learning on learning efficiency, learning retained an independent contribution to improved efficiency. Squirrels that used integrative tactics more made less total errors than squirrels that used less; suggesting learning a task relevant tactic using spatial cues can provide direct benefits in maximising rewards and minimising time costs.

Keywords: reversal learning, experience, strategy, squirrels, flexibility.

INTRODUCTION

Learning provides a mild form of flexibility by which individuals can adapt their behaviours according to environmental demands or changes (van Schaik, 2013). The occurrence of learning, as argued by Dukas (2013), confers advantages on a variety of fitness measures. Such ultimate gain for fitness presumably outweighs the inevitable time and effort costs of the learning process, especially when the demand or change recurs. Hence, it is important to understand the learning process, in particular how animals learn and how they learn '*how to learn efficiently*' (Harlow 1949, p.51) under such recurring changes.

To assay flexibility in the learning process under recurring change, investigators have often used discrimination reversal learning (Shettleworth 2010, p. 210-211). Pavlov (1927) introduced the reversal learning paradigm, in which the reinforcement contingency switches between two stimuli. In the acquisition phase, individuals need to associate one of two stimuli with a reward (A- B+). Once they reach a predetermined learning criterion, the reinforcement contingency is switched, for a reversal phase in which the previously unrewarded stimulus becomes rewarded while the previously rewarded stimulus becomes unrewarded (A+ B-). In *serial* reversal learning, the reinforcement contingency repeatedly switches between the two stimuli (e.g. Mackintosh & Cauty, 1971). Under such recurring change, a wide range of species (Warren, 1965, 1974) have been shown to reduce the number of errors across successive reversals. Such a trend indicates that individuals have increased their learning efficiency, thus increasing the reward gain and reducing the time cost, with cumulative experience (Flaningam, 1969). Harlow (1949) called such gains in learning efficiency across repeated tasks of the same type 'learning set' or 'learning to learn'.

So, how do animals improve their efficiency under recurring change? Previous research has consistently shown that attention and memory are the key factors (Shettleworth, 2010).

Attention. Selective attention to the rewarded cue (e.g. Mackintosh, Brendan, & Valerie, 1968; Mackintosh & Little, 1969) or to local feedback (e.g. Rayburn-Reeves, Stagner, Kirk, & Zentall, 2013) is an important factor in completing the reversal learning task more efficiently. Appropriately directed attention allows animals to achieve efficiency by making associations between the relevant cues and the rewards, but this is more likely to happen if the experimental design is ecologically relevant to the study species. For example, bumblebees associate olfactory cues with food rewards in an olfactory reversal paradigm (e.g. Mota & Giurfa, 2010) and rats associate extra- or intra- apparatus distance cues with the goal in a spatial learning task (e.g. Kraemer, Gilbert, & Innis, 1983).

Memory. Previous memories are certainly not completely erased by new experience. However, the influence of previous memories on learning efficiency is not necessarily positive. On the one hand, improved retention of information within the current phase is implied if individuals learn the reversal faster than the initial acquisition phase (e.g. Calhoun & Handley, 1973; Chittka, 1998). On the other hand, memories from the previous phase can proactively interfere with individuals' performance on the current task (e.g. Chittka, 1998; Mackintosh et al., 1968; Strang & Sherry, 2014, but also see Raine & Chittka, 2012).

These factors, however, concern the mechanisms involved in learning each reversal task; they do not as such allow for the formation of learning set, or any other form of increased flexibility in the learning process. Increased flexibility during the course of learning

could, however, result from a change in the response strategies or tactics that an individual uses in solving a task.

Tactic change. A tactic can be considered as a specific behavioural pattern that an individual shows in responses to a task. The best illustration of how changing of tactics could increase efficiency comes from the typical two stimulus serial reversal task; the most efficient tactic for maximising reward gain under this design is the 'win-stay, lose-shift' (WSLS) strategy (Shettleworth, 2010). In WSLS, individuals follow the same stimulus if it is immediately rewarded (win-stay) and shift to the alternative stimulus following non-reinforcement (lose-shift). Depending on the reversal paradigm, the manifestation of such behavioural pattern may reflect that the individual has learned an associative rule between a stimulus and a reward as in two stimulus serial reversal task, has formed a spatial relationship between the rewarded locations as in spatial pattern learning (e.g. Brown & Terrinoni, 1996; Brown, Zeiler, & John, 2001), is using intra- and/or extra- apparatus cues to remember rewards individually, or any combination these of mechanisms. Although learning a tactic such as WSLS should lead to improved efficiency, it does not happen immediately. The formation of a task efficient tactic over the course of learning is progressive, as it gradually replaces the trial-and-error tactics employed at the start. In learning paradigms other than two-stimulus reversal, individuals may employ more than one tactic in a given learning phase, which allows us to measure change of tactics by observing the proportions in which given tactics are exhibited during the course of learning. Learning an appropriate tactic for a task has been shown to be advantageous for solving the same problem in future occasions even if individuals no longer remember the specific task information (Bonney & Wynne, 2002), or if specific task information becomes misleading, as it does in reversal tasks. This evidence

suggests that learning a tactic is one way in which individuals can *'learn how to learn efficiently'* (Harlow, 1949).

In the present study, we first examined whether Eastern grey squirrels (*Sciurus carolinensis*) would show a learning set in the serial reversal task. Previous studies of learning set in the *Sciuridae* family have used successive discrimination learning tasks, in which the same reward contingency is applied to a new pair of stimuli in each discrimination phase (e.g. Harlow, 1949). In this task, fox squirrels, *Sciurus niger,* and round-tailed ground squirrels, *Citellus tereticaudus,* failed to learn any task after the first discrimination phase (Flaningam, 1969; Rees, 1968). However, the response strategies that are readily learned are likely to be those that are ecologically relevant to the species in question (e.g. Day, Crews, & Wilczynski, 1999; Liedtke & Schneider, 2014; Mota & Giurfa, 2010), and these studies all used discrimination of objects (e.g. small toys or jewellery), which is not an obviously ecologically relevant ability for sciurids. To accommodate squirrels' natural learning style, we utilised spatial learning, which is certainly ecologically relevant for Eastern grey squirrels since they are scatter hoarders. Squirrels were required to remember which two of four locations contained food (see *Methods*). The four locations were arranged in a square and the two rewarded locations were always at opposite ends of a diagonal; reward contingency was only switched between the two diagonal pairs of wells across phases. We recorded the sequence in which the squirrels visited them, so that we were able to categorize the sequences as resulting from two different types of tactics, sequential search tactics (Fig. 1a) and integrative search tactics (Fig. 1b). Under this set up, the efficient way to maximise the gain and minimise the time costs was to use integrative search tactics. If squirrels formed a learning set in this situation, we would then be able to examine how they achieved the improved efficiency across the reversal phases and the possible cognitive processes

underlying the factor(s), in particular whether changing to integrative tactics made a contribution. Figure 2 illustrates the predictors that would potentially affect the learning efficiency in this serial reversal task. We examined how each predictor varied across the learning process and their relationship to learning efficiency.

We chose grey squirrels as a study species because field studies have shown that they adjust their food protection tactics flexibly under intra-conspecific food competition (Hopewell & Leaver, 2008; Hopewell, Leaver, & Lea, 2008; Leaver, Hopewell, Caldwell, & Mallarky, 2007; Schmidt & Ostfeld, 2008; Steele, et al., 2008), so it is reasonable to suppose that this species would also show flexibility in spatial learning. Also, grey squirrels are scatter-hoarders and cache thousands of nuts every year (Thompson & Thompson, 1980), so the number of locations they were required to remember in this task should not pose a problem for them. Moreover, although there is currently limited evidence indicating how squirrels remember cache locations and status, it is clear that they have an accurate memory of the locations of their caches (Jacobs & Liman, 1991; Macdonald, 1997) and they can update this memory to reflect the current state of each cache (unused, used, or pilfered). Finally, given that the food preferences of grey squirrels imply that they tend to maximise energy gain (Smith & Follmer, 1972), the use of a highly preferred food reward should lead to rapid learning.

Figure 1

Figure 2

METHODS

Subjects

Five captive squirrels (three males and two females) housed at the University of Exeter were used in this study. They were housed in large cages, from which they could be

given access to the test room via an overhead mesh tunnels controlled by sliding doors (for full details, see Hopewell, Leaver, Lea, & Wills, 2010). Accordingly, the squirrels were not handled directly in the experimental procedures or normal husbandry. They were not food deprived during the experiment. Water was provided *ad libitum* and their daily diet included fresh fruit, pumpkin seeds, sunflower seeds, dried vegetables and tiger nuts. Data collection was from Mar-July, 2014. Experiments were conducted when squirrels were most active, usually during 0700-0900 and 1400-1700. This study was approved by the Ethical Review Group at the University of Exeter. Squirrels were treated in accordance with Association for the Study of Animal Behaviour guidelines on animal welfare and UK law.

Apparatus

Fig. 3a and 3d show the apparatus (hereafter, the poke box). It was a square wooden box (dimension: 21cm x 21cm x 4.5cm) composed of four layers. Layers (from top to bottom) consisted of an aluminium plate (21cm x 21cm x 0.5cm, length x width x depth), a wooden *upper* container (21cm x 21cm x 1.7cm), a piece of metal mesh (21cm x 21cm x 0.5cm), and a wooden *base* container (21cm x 21cm x 2.7cm). The entire assembly was secured with wing-nuts. As Fig 3b shows, the upper and base containers had 16 food wells (each was 4.5 cm in diameter), with four wells in a row and divided by the metal mesh. As Fig. 3c shows, the metal plate had 12 holes of the same diameter, corresponding with the food wells.

Figure 3

Procedures

Pre-training

Squirrels went through standardized pre-training before the main experiment. In the pre-training, we used all 12 food wells. Cheerio pieces (Nestlé® Cheerios Cereal) or pine nuts (according to the known food preference of each squirrel) were placed in the base container of each well as a control for olfactory cues. We covered these baits with the metal

mesh so that squirrels could smell but could not eat the food. We then baited the upper container with accessible food rewards, either one-third of a Cheerio or one pine nut per food well. A sheet of aluminium foil (21cm x 21cm) and a sheet of white paper (21cm x 21cm) were placed between the upper container and the metal plate. This aimed to further minimise the chance of squirrels using olfactory cues in locating the hidden food.

Pre-training was divided into four stages: opened-well stage (habituation), crossed-stage, diagonal-cut stage, and closed-well stage. The first stage aimed to encourage squirrels to come close to the apparatus and to obtain food from the wells. No foil was used and the paper had holes corresponding to the food wells. In the next two stages, the crossed-stage and diagonal-cut stage, we aimed to allow squirrels to gain experience of peeling off the paper or using their front paws to scratch to open the wells by themselves. No foil was used in these stages. In the crossed-stage, there were two perpendicular diagonal cuts in the paper above each food well. In the diagonal-cut stage, a single cut was used and the diameter of this cut was gradually reduced. In the final stage, the closed-well stage, foil was introduced and the paper was not cut, so that the squirrels had to open the wells either by their claws or teeth. Pre-training was conducted on alternate days with three trials per pre-training day, and each trial lasted for a maximum of 10 minutes (30 minutes in total per training day). The first trial of each day was a repeat of the previous training stage. Individuals advanced to the next training stage after they had successfully obtained all baits across three trials. At the end of each trial, we slowly approached the testing squirrel, removed the poke box, and re-baited the food wells outside the test room.

Training

The same poke box and a similar procedure to that in the pre-training stage were used in the training phase. The training phase used only the four wells at the corners of the poke box (the other wells were capped) and we changed the food reward to hazelnuts or cashews,

depending on each squirrel's preference, to increase their motivation. Two half hazelnuts were used for four squirrels and two pieces of cashews were used for one squirrel. As in the pre-training phase, we controlled for olfactory cues by first baiting all four base wells with the corresponding food rewards, either hazelnuts or cashews, under the mesh. As shown in Fig. 3c, we minimised any side preference by placing baits in diagonally opposite wells while the wells on the opposite diagonal were empty. We further minimised olfactory cues by placing two sheets of foil, instead of a single sheet, between the metal plate and the upper container and rotating the box randomly between trials. Fig. 3d shows the poke box as finally prepared for the training phase. The poke box was then put in the centre of the test room so as to equalise the distance between the box and the corners of the testing room. The sides of the box were always parallel to the walls of the testing room, and well numbers were defined in terms of their location relative to the testing room, e.g. well 3 was the one nearest to the corner between the side wall and the door. Squirrels could therefore use structures in the test room as extra-apparatus cues to identify the rewarded wells.

There were 12 phases in total (an acquisition phase and 11 reversals). We tested one squirrel at a time and pseudo-randomized which diagonal pair of wells (either wells 1 and 3 or wells 2 and 4, Fig. 1a and 1b) was positive for a squirrel in the acquisition phase. The learning criterion was three consecutive correct trials. Correct trials were those in which squirrels obtained food from both of the rewarded wells as their first and second choices, without choosing any non-rewarded wells before or between choices of the rewarded wells. At each reversal, both wells that had previously been rewarded became non-rewarded and vice versa.

A trial started when a squirrel approached the poke box. A well selection was indicated by the squirrel tearing the corresponding paper and the foil sheet. The trial ended when the squirrel moved 25 cm away from the poke box or had not obtained a reward for 10

minutes. Squirrels received a maximum of four trials each day, depending on their motivation. All the behavioural responses were captured by a video camera (Panasonic SHD-90) that was set adjacent to the cage.

To minimise the possibility of squirrels learning the location of rewards from direct observation, the experimenter (the first author) approached the box quietly and removed it for re-baiting outside the test room after each trial. As squirrels can use odour cues to locate caches (Jacobs & Liman, 1991; Macdonald, 1997), we randomised the orientation of the poke box for the next trial to avoid any odour cues being left on the poke box which might aid in locating rewards. We then applied disinfectant on the poke box using wipes after we re-baited the wells so as to minimise any odours left by the experimenter. The whole set up procedure did not last longer than two minutes. The next trial began after the experimenter quietly approached the test cage and placed the poke box in the centre of the test cage. We re-applied the disinfectant procedure before the next squirrel was tested. This aimed to minimise the scent that the previous squirrel left on the poke box, which might affect the decision making of the next individual tested.

Measurement

Learning efficiency. We measured learning efficiency by the number of errors (trials in which a squirrel opened either unbaited well before or between opening any baited wells) that a squirrel made in each phase.

Proactive interference. To examine whether squirrels' performance was affected by proactive interference from the previous reward contingency, we counted the number of non-rewarded first choices across trials and divided this number by the total number of trials.

Learning tactics. To examine the tactics that a squirrel employed to learn the task, we recorded the sequence of wells that the squirrel chose in each trial for each phase. Each trial was categorized as using either sequential or integrative tactics. Fig. 1a shows examples of a

sequential tactic, in which squirrels made choices in clockwise or anti-clockwise directions with no diagonal transitions. Fig. 1b shows examples of an integrative tactic, in which the squirrels followed a diagonal direction between two choices. Amongst the tactics, only the left-panel of integrative tactic of Fig. 1b shows the most efficient tactic for this task, and this was accordingly considered as the correct response. Incorrect responses could be made while using either of the tactics: Fig. 1a shows how the incorrect responses could be made by a sequential tactic while the right-panel of Fig. 1b shows how the incorrect responses could be made by using an integrative tactic. We further calculated the proportion of integrative tactics used in each phase by dividing the total number of integrative tactics (both correct and incorrect) by the total number of trials taken in each phase. This calculation included the last three (criterion) trials, in order to include the data from one squirrel that showed no errors in two phases.

Other interference information. We included a measure of possible interference information that might affect learning efficiency. In each correct trial we counted the number of wells that the squirrels opened after opening the rewarded wells. We then divided the total number of extra wells opened by the number of correct trials in each phase to obtain the rate of irrelevant behaviours induced by interference for each squirrel.

Data analysis

Page's trend test (Page, 1963) was used to test the change in learning efficiency across phases. Wilcoxon signed rank tests were used to examine whether squirrels took more trials to learn one of the diagonal pairs rather than the other and to compare the number of errors in the first reversal phase with the acquisition phase.

To assay proactive interference, binomial tests were applied to each individual. We tested whether the proportion of first choice preferences for a rewarded well was different from the 50% expected by chance in the acquisition phase and the first reversal phase. To minimise any possible bias, the first trial and the last three criterion trials of each phase were excluded, as the first trial in the acquisition phase was a random choice and first trial in each reversal phase was immediately affected by the previous contingency.

For each squirrel in each phase, we obtained the proportion of trials in which integrative tactics were used, and we used Page's test to examine the trend in the proportion of integrative search tactics employed across phases. We used a Spearman's rank correlation coefficient to examine whether the squirrels' tendency to use integrative tactics was correlated with its overall number of errors in completing the reversal task.

To assay the hypothetical model in Fig. 2, a Generalized Estimating Equations (GEE: Hardin & Hilbe, 2003) analysis with exchangeable correlations was used. GEE is a pseudo-parametric test that uses robust variance to estimate population-averaged effect as well as considering the individual correlations under repeated measures. It has been proven to be a robust statistics for datasets with extreme small-sample size and comprised entirely of repeated measures, as in our case (Wang & Long, 2011). As GEE modelling with small samples can underestimate the true variance of the sample, we applied Wang and Long's (2011) adjusted variance in the GEE models. Table 1 shows the covariates and the corresponding measurement used for the GEE models. To compare the effect size of the predictors, we standardised the covariates (phase number, tactic change, proactive interference and other irrelevant interference behaviours) in each phase, but not the dependent variable, learning efficiency. We used the Poisson distribution for count dependent

variable, learning efficiency, and the Gaussian distribution for other continuous dependent variables (e.g. tactic change). We did not test any interactions so as not to exhaust the degrees of freedom. R version 2.15.2 (R Development Core Team, 2012) was used to analyse the data; the 'gee' package was used to apply GEE (Carey, 2015), and the 'crank' package was used to apply Page's trend test (Lemon, 2014). All the tests were two-sided with significance level as $\alpha = .05$.

Table 1

RESULTS

Learning efficiency

All the squirrels completed 11 reversals. Fig. 4 shows that individual squirrels made fewer errors as the 12 phases progressed, and this trend was significant (Page trend test: $\chi^2(1)=18.31$, $p<0.001$). Squirrels did not require more trials to learn one diagonal pair of wells than the other (Wilcoxon signed rank test: $p=0.313$), nor did they require more trials to learn the acquisition phase than the first reversal phase (Wilcoxon signed rank test: $p=0.625$).

Figure 4

Proactive interference

Fig. 5a shows that when the first trial and the last three criterion trials of each phase were excluded, the proportion of squirrels' first choices in each trial for one of the rewarded wells was greater than chance in the acquisition phase, and this trend was significant (77%; Fisher's pooled: $\chi^2(10)=65.43$, $p<0.001$). Fig. 5b shows that when the first trial and the last three criterion trials were excluded, the proportion of trials on which squirrels' first choice for any of the rewarded wells was also significantly greater than chance in the first reversal phase (69%; Fisher's pooled: $\chi^2(10)=20.56$, $p<0.02$).

Figure 5

Tactic change

Fig. 6a shows that the proportion of integrative tactics used increased across phases, and this trend was significant (Page trend test: χ^2 (1)=8.11, p<0.005). The mean proportion of integrative tactics used by a squirrel across all phases was positively correlated with the overall errors it made to complete all the reversals (r_s=0.7), but this result was not significant. Fig. 6b shows the proportion of first trials across 12 phases on which the squirrels used sequential and integrative tactics. Overall, squirrels tended to use the sequential tactic rather than the integrative tactic on the first trial of each new reversal (Fisher's pooled: χ^2 (10)=23.88, p<0.01); however, one squirrel, Suzy, used the integrative tactic more often than the sequential tactic, although this trend was not significant (binominal test: p=0.388).

Figure 6

Predictors of learning efficiency

Table 2 shows the results of GEE modelling. The number of errors made in a phase decreased across reversals, decreased with proportion of integrative tactics, increased with the amount of proactive interference, and decreased with the amount of other irrelevant interference. Only phase number (p<0.001) and tactic change (p=0.025) showed significant effects. The effect of other interference also approached significance (p=0.057) but the effect of proactive interference did not (p=0.197).

Table 2

Table 3

Effect of learning on proactive interference, tactic change, and other interference information

Following the hypothetical model in Figure 2, we tested whether phase number had an effect on tactic change, proactive interference and other interference information, and therefore whether any of these variables could be mediating the effect of phase number on efficiency.

Table 3 shows the result of the GEE model: only phase number was significantly related to tactic change ($p<0.001$). Hence, the proportional use of integrative tactics increased across phases. However, even with tactic change included in the model, there was still a significant effect of phase number on efficiency. Tactic change therefore partially mediated the effect of phase numbers.

DISCUSSION

We examined whether squirrels would form a learning set in a spatial reversal learning task and if so, how squirrels achieved this improved efficiency, by examining the variation of four potential predictors, learning (phase number), proactive interference, tactic change, and other irrelevant interference information across phases. The discussion here focuses on how the squirrels appear to be '*learning how to learn efficiently'* (Harlow, 1949), particular attention is paid to the role of tactic change in the learning process in relation to improved learning efficiency. We also discuss the possible cognitive processes that are involved in the tactic change under this specific design and the response strategies in respect to squirrels' ecological behaviours. Our results showed that both accumulated experience and tactic change led to increased efficiency. Although the effect of learning on efficiency was partially mediated by tactic change, its significance was not completely negated when tactics were included in the model.

Firstly, our results showed that the squirrels did form a learning set (Harlow, 1949), since they showed clear improvement over successive reversals, becoming more efficient in adjusting their behaviour to the recurring change in reward contingencies (Fig. 4). Our result is apparently contrary to previous studies in which other species in *Sciuridae* family have failed to improve over successive object-discrimination task (eastern fox squirrels, Flaningam,

1969; round-tailed ground squirrels, Rees, 1968). It is possible that the difference of results may be due to the different methodology used in the serial reversal *versus* the successive discrimination tasks, but it is also important to consider that our use of a spatial task, which is certainly ecologically relevant to squirrels, as scatter hoarders, plays an important part in the squirrels' ability to engage with the task.

Secondly, we examined the process by which squirrels were '*learning how to learn efficiently'* (Harlow, 1949). Our results showed that the simple accumulation of experience has the greatest effect amongst the variables. Apparently, experience may allow squirrels to become familiar with the recurring change. Individuals did not use more trials or make more errors in the first reversal phase than in their acquisition phase. It appears that squirrels may be predisposed not to rely on previous information, given that our model shows that neither proactive interference nor other irrelevant information is a significant predictor of learning efficiency. It is also notable that squirrels visited at least one of the two rewarded locations as their first choice significantly more often than the non-rewarded locations in the first reversal phase (Fig. 5b). These results suggest that squirrels quickly learn to adapt to the change of contingency, allowing current reward information to override memories of past contingencies. Altogether, the evidence supports the idea that learning to be flexible can have adaptive significance in fitness measures (Dukas, 2013), here, we show that learning provides direct advantages in maximising reward gain as well as minimising time cost to achieve learning efficiency.

As we predicted, another significant predictor of increased learning efficiency was tactic change. The tactics that animals use in a task may reflect the formation of an abstract rule about the alternating pattern of reward contingency across the phases. In our case,

squirrels would form a diagonal rule between the two paired rewards (i.e. pick the diagonally opposite well if a rewarded well is found) and apply it in each phase to increase efficiency. Our results seem to support this explanation, given that the squirrels changed the tactics they used *within* phases and by the end of the experiment, some squirrels were making zero, one or two errors before reaching criterion (Fig. 4). However, detailed analysis of the tactics used in the first trials after a contingency switch makes us question whether this explanation is complete. If squirrels have learned the rule, they should make the integrative tactic errors that are similar to the right panel of Fig. 1b in the new reward contingency. But this did not happen: as shown in Fig. 6b, squirrels reverted to sequential tactics at the beginning of each new phase, even for the individuals that reached the criterion with one or two trial errors. These results suggest that squirrels do not become efficient by forming the diagonal rule. Instead, results suggest that this integrative tactic is implemented by learning the spatial pattern of the reward locations, perhaps through the use of extra-apparatus cues. Although we have no evidence for the formation of spatial pattern in our case, the use of extra-apparatus cues is possible for two reasons: firstly, the apparatus was always parallel to the walls of the test room, which then provide unique information for squirrels to locate the reward. Secondly and more importantly, if squirrels could only use spatial pattern for this task, then first choice between rewarded and non-rewarded wells should be at chance level, as happened with rats in the pole box experiment (e.g. Brown & Wintersteen, 2004). However, squirrels located one of the rewarded wells significantly more than the non-rewarded wells as their first choice both in the acquisition phase (Fig. 5a) and the reversal phase (Fig. 5b), and this clearly shows that they relied on more than internal spatial representation to locate the reward. The use of extra-apparatus use have also been shown in other members of the *Sciuridae* family such as northern flying squirrels (Gibbs, Lea, & Jacobs, 2007) and fox squirrels (Waisman & Jacobs,

2008), and these species were found to be flexible in cue use and use more than one frame of reference in remembering the locations of rewards.

Tactic change may be related to increased efficiency because it is associated with attention. Attention to cues and local feedback have been suggested to be important for reversal tasks (e.g. Mackintosh et al., 1968; Mackintosh & Little, 1969; Rayburn-Reeves et al., 2013). Although a serial reversal task puts a premium on attention to the recent rewards received, here we also suggest that attention to extra-apparatus cues is useful with stable reinforcement contingencies.

Our results highlight the advantages of changing tactics in response to the task demands so as to increase learning efficiency, maximise energy gain and minimise time cost. Although the correlation result was not significant, individuals that used integrative tactics made fewer total numbers of errors across phases than individuals that used this tactic less, and they thus secured the same number of rewards at a lower cost of time and effort. This trend confirms our expectation that changing tactics in the learning process brings advantage. The apparent variation of our squirrels in how soon they switched to use integrative tactics may suggest that there is variation in intrinsic learning ability, with some squirrels requiring more trials to memorise the reward value of each well, whereas others reached the criterion with no or only a single error trial. However, given that all locations had contained rewards some of the time, depending on the reinforcement contingency in force in a particular phase, squirrels that preferred to use the sequential search behaviour might not be making ‘errors’ but instead using an alternative strategy in foraging (Evans & Raine, 2014), involving a different speed/accuracy trade-off (Chittka, Dyer, Bock, & Dornhaus, 2003) in the face of a complex design (Cakmak et al., 2009), even if the time cost of sequential tactics is higher

than that of integrative tactics. A quick but inaccurate foraging style has been shown to be adaptive in some foraging situations (Burns, 2005).

Although the cost of making an 'error' is small in this design, the fact that squirrels significantly increased their proportion use of integrative tactic *within* each phase shows that they were motivated to increase efficiency in obtaining the hidden rewards (Fig. 6a). Grey squirrels have the capacity to re-locate their caches within 5 cm accuracy (Macdonald, 1997), and in field condition searching at random could be less efficient than relying on memory and using appropriate search tactics. Our squirrels' preference for using sequential search tactics in the first trial of a new reward contingency (Fig. 6d) – that is, in response to a failure to obtain expected reward may be an example of an ecologically driven tendency in response tactics towards change, as in lizards (Day et al., 1999), honeybees (Mota & Giurfa, 2010) and jumping spider (Liedtke & Schneider, 2014). Squirrels may consider the distance between food locations during foraging or cache retrieval, and they will initiate a search in adjacent locations that are around the remembered cache location when search in the expected place for a cache fails. Hence, if well 1 is unexpectedly empty, the likelihood of a squirrel to search in well 2 and 4 is higher than well 3, as both well 2 and 4 are closer to well 1 (13 cm) than well 3 (21.7 cm). Such a search in an adjacent location may be a more natural response, and a more efficient one under natural conditions, than moving to the diagonally opposite well. Hence, both the search tactics we considered are ecologically relevant and may have adaptive advantages. Future research could look at these possibilities by using a larger poke box which then allows squirrels to reveal their response strategy with rewards that are hidden further apart.

In conclusion, we provide the first evidence that squirrels increase learning efficiency with repeated exposure to changing reward contingencies in an ecologically relevant task, and furthermore we have been able to show how they achieve this. Squirrels rapidly form a learning set after experiencing successive reversals. This rapid decrement in errors is predicted by increased experience but is accelerated if they are flexible enough to change tactics under the recurring change of contingencies. This is the kind of cognitive capacity that should be useful to a scatter-hoarding animal, which needs to return efficiently to cache sites to empty them, but thereafter to avoid wasting time on revisit to sites that have been emptied or found to be pilfered.

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